Measurement of reactive oxygen species in cultured primary rat hepatocytes

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Organisation

Name of the organisation Vrije Universiteit Brussel (VUB)

Department Pharmaceutical and Pharmacological Sciences

Specific Research Group or Service In Vitro Toxicology and Dermato-Cosmetology

Country Belgium

SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Rat
Type of cells/tissues/organs	Hepatocytes

DESCRIPTION

Method keywords

Hepatocytes

Hepatotoxicity

cells

rat

rodent

ROS

reactive oxygen species
DCFH-DA

Scientific area keywords

hepatic toxicity
hepatology
hepatocytes
toxicity testing
toxicity

Method description

Basically, the standard operating procedure outlined in this document consists of the following steps, namely, (i) preparation of the cells for the DCFH-DA assay, (ii) DCFH-DA test procedure, (iii) processing of the results. Practical details are provided for each of these steps and are followed by some useful tips based upon our own hands-on experience. The fluorescent assay DCFH-DA is based on the ability of the non-fluorescent lypofilic DCFH-DA probe to penetrate viable cells, where it is being deactylated by the presence of intracellular esterases and trapped within the cells. Upon exposure of the cells to a stimulus that generates the oxygen metabolic burst and subsequently the production of ROS, the probe is oxidized and starts to emit an intense green fluorescence. This assay allows the determination of the oxidative stress induction potential of chemical substances. As such, after incubation with the probe, the cells are exposed to the selected substances and the respective emitted fluorescence can be measured for a period of 30 min. The increase in fluorescence is proportional to the amount of ROS that are being formed within the cells. The results can be read by the use of a multiwell scanning fluorimeter (plate reader) using an 485-495nm emission filter and an 520-530nm excitation filter. ROS production is expressed as a ratio of treatment versus control. This method provides a sensitive, reproducible and integrated signal of both attached cells and cells in suspension.

Lab equipment

Multiwell scanning fluorimeter (plate reader) using an 485-495nm emission filter and an 520-530nm excitation filter ;
Laminar flow cabinet ;

Thermostated bath.

Method status

History of use
Internally validated

PROS, CONS & FUTURE POTENTIAL

Advantages

The method is sensitive, reproducible and capable of giving an integrated signal of both attached cells and cells in suspension.

Challenges

Unequally seeded cells can give divergent testing results. Take care to handle the seeded cells with caution. Air bubbles in the wells of the 96-well plate can cause negative testing results, gently shake the plate before putting it into the plate reader to avoid this.

Future & Other applications

Can be applied to other cell types ;
Possible modification needed.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

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Associated documents

ROS assay.doc